Reference: *Biol. Bull.* **207:** 116–129. (October 2004)

Microscaling: Why Larger Anemones Have Longer Cnidae

LISBETH FRANCIS

Shannon Point Marine Center, Western Washington University, 1700 Shannon Point Rd., Anacortes, Washington 98221-4042

Abstract. Scaling analysis provides a quantitative method for describing and comparing how qualities of organisms vary as a function of body size. However, cell level phenomena have been notoriously hard to analyze because animal cells and organelles have such irregular shapes. The intracellular cnidae make good models of scaling at the cell level because they are durable and easy to image and measure. The mean length of unfired tentacle cnidae (spirocysts) varies continuously, and reversibly, with body size for three macrophagous anemone species. Significant differences in spirocyst shape and size relative to body mass are related to differences in tissue functions and species ecologies, strongly suggesting that cnida size, shape, and scaling patterns respond to natural selection. Cnida scaling patterns can be treated as features of cnidarian life histories. Spirocyst scaling exponents (slopes of log cnida dimension *vs.* log body weight) are similar to each other (0.05–0.09) and to reported values for animal somatic cells (0.017–0.17), but are much smaller than reported values for anemone basal diameters (0.30–0.38). I propose, here, a general, mechanical explanation for microscaling of structural secretory cells and their secretions, including the cnidae. Larger bodies require thicker, pliant sheets of sluggishly respiring extracellular support materials such as mesoglea and basement membrane. Thicker mesoglea can support larger, taller epithelial cells, which in turn provide additional maintenance services for these progressively thicker acellular layers. Ultimately, larger, taller cells can secrete and support larger, longer cnidae.

Received 31 July 2003; accepted 7 July 2004.

Abbreviations: b, slope of the log-log plot; OLS, ordinary least squares regression estimate of line fit; RMA, reduced major axis estimate of line fit; 95% CI, 95% confidence intervals (for slope and intercept estimates); %SEE, percent standard error of estimate.

Introduction

Although many organisms exhibit a wide range of adult body sizes, remarkably little is known about how cell size or organelle size varies (scales) with body size, or about the adaptive significance of such variation. Cnidae—structurally and functionally unique organelles of cnidarians (Mariscal, 1984) that include spirocysts, nematocysts, and ptychocysts—are particularly tractable for studying such microscaling because they have comparatively simple shapes and are much easier to image and to measure than entire cells and organelles. Cnidae are distinctive and morphologically variable microtools composed of a durable cartilage-like material (Blanquet, 1988) secreted inside a single cell. Mechanically, they are pressurized capsules with an attached eversible tubule that is inverted inside the capsule before firing. Either the capsule or its tubule may be filled with toxins, irritants, or adhesives that are released when the tubule everts during firing.

In spite of many quantitative studies of cnida size (Stephenson, 1929; Chintiroglou, 1996; Chintiroglou and Simsiridou, 1997; Chintiroglou *et al.*, 1996, 1997; Chintiroglou and Karalis, 2000; Zamponi and Acuña, 1991; Acuña and Zamponi, 1997; Williams, 1996, 1998, 2000), changes in cnida size with body size appear to have been overlooked or discounted; and no studies have been conducted on cnida scaling *per se*. In the taxonomic literature, tissue-specific size ranges for each cnida population have been treated as species-specific characters (discussed in Fautin, 1988), although a possible relationship between developmental stage or polyp size and cnida size has occasionally been noted (Cutress, 1955; Sebens and Laakso, 1978; Fautin *et al.*, 1989; Ryland *et al.*, 2004).

Sea anemones (order Actiniaria) make good subjects for a scaling study for four reasons. First, body size, rather than age, appears to be the most critical determinant of the life

history in this group. Second, growth is reversible: under favorable conditions anemones grow and under unfavorable conditions they shrink, so size and age are essentially decoupled (review in Shick, 1991). Third, in clonal species that aggregate (*e.g., Anthopleura elegantissima;* Francis, 1973a), a full range of stages may occur simultaneously, providing a morphologically diverse array of individuals cloned from the same zygote (Francis, 1976). Finally, among anthozoans, sea anemones have evolved the greatest variety both of cnidae and of polyps, with the highest level of regional specialization (*i.e.,* different sizes and types of cnidae in different parts of the body), resulting in substantial between-species variability (Schmidt, 1974; Weill, 1934 a, b). This variation within and among species allows strong comparative tests relating cnida scaling to species habitats and tissue functions.

I chose spirocysts for this first study of cnida microscaling for several reasons. First, spirocysts are easy to recognize. Second, they are an abundant, widespread, unique, and probably monophyletic cnida type, showing little evolutionary divergence in form within the Hexacorallia (Schmidt, 1974) and no major morphological differences among the actiniarian anemones (Rifkin, 1991). Third, in members of the genus *Anthopleura,* spirocysts occur in two tissues that have quite different functions: tentacles that are used mainly in feeding, and acrorhagi that are specialized, inducible structures (Francis, 1976) used only in territorial battles (Francis, 1973b), most often with other anemones (Francis, 1985). Finally, Williams (1996, 1998, 2000), who developed a standardized protocol for sampling, reporting, and testing cnida size variation, has confirmed that spirocysts from *Metridium* showed higher than usual coefficients of variation (Williams, 2000), validating a general impression that within-sample variability is higher for spirocysts than for other cnidae.

Here I describe scaling of one adhesive cnida type (spirocysts) from two different tissues (ectoderm of the feeding tentacles and acrorhagi) of two sympatric anemones that have contrasting diets, growth forms, and social structures. *Anthopleura elegantissima* (Brandt, 1835) dominates large areas of the mid-intertidal on rocky shores by replicating asexually to form dense, segregated clonal groups (Francis, 1973a). It uses the inducible acrorhagi to attack and repel all other anemones except clonemates (Francis, 1973b). While *A. elegantissima* eats primarily plankton, invertebrate larvae, and smaller intertidal invertebrates, *Anthopleura xanthogrammica* (Brandt, 1835) commonly eats larger intertidal invertebrates, including dislodged mussels and barnacles (Sebens, 1981a). *A. xanthogrammica* develops larger polyps than *A. elegantissima* (Sebens, 1981b), does not replicate asexually, and exhibits less aggression against conspecifics (Sebens, 1984). Data for the tentacle spirocysts of *Tealia crassicornis* (Mueller, 1776) (a closely related aclonal species without acrorhagi), are included for contrast, because this species captures larger and more active prey—including moderate-size crabs, snails, and mussels (Sebens and Laakso, 1978)—than is typical for species of *Anthopleura.*

Finally, I found differences in spirocyst size among tissues and species to be consistent with apparent differences in the selective regimes; however, increase in spirocyst size with body size was similar for all populations, with scaling exponents similar to those reported for cell size variation within and among animal species (Munro, 1969; Munro and Gray, 1969; Maldonado *et al.,* 1974; Peters, 1983; Calder, 1984; Stevenson *et al.,* 1995). To account for these unusually small scaling exponents, I introduce a possible mechanical explanation for microscaling at the cellular level. Cnida scaling (and the scaling of cellular secretions, generally) may typically reflect the underlying phenomenon of cell scaling.

Materials and Methods

Specimen collection and handling

Specimens of *Anthopleura elegantissima* were from Eagle Point and Eagle Cove, San Juan Island, Washington (48°28′N, 123°03′W). *A. xanthogrammica* specimens were all from Charleston, Oregon (43°20′N, 124°20′W); and specimens of *Tealia crassicornis* were from Anacortes (48°00′N, 131°01′W), and San Juan Island, Washington. Anemones were weighed alive by first removing any clinging debris, and then blotting them firmly to cause contraction and to remove all expressed moisture. This method is non-destructive and provides a practical basis for comparing the sizes of soft-bodied animals such as anemones. Daily measurements of 11 individuals (*A. elegantissima*) over a 3-day period indicated that wet weights were reproducible within ± 3%.

Within 2 weeks of collection, most animals were either examined alive, or preserved in 10% formalin after first anesthetizing them in seawater by adding an equal volume of magnesium sulfate solution (7.5% by weight of common Epsom salts).

Anemone selection

To determine whether average size of the tentacle spirocysts is a function of body size for the clonal aggregating anemone, *A. elegantissima,* I collected individuals spanning the range of sizes found within a clonal aggregation at Eagle Cove, Washington. Recently divided animals with obvious fission scars were always excluded. To compare the scaling of acrorhagial and tentacle spirocysts, I collected one or two individuals with well-developed acrorhagi from seven separate clusters of *A. elegantissima* in densely populated tidepools at Eagle Point, Washington.

To compare scaling patterns for aclonal species, I also

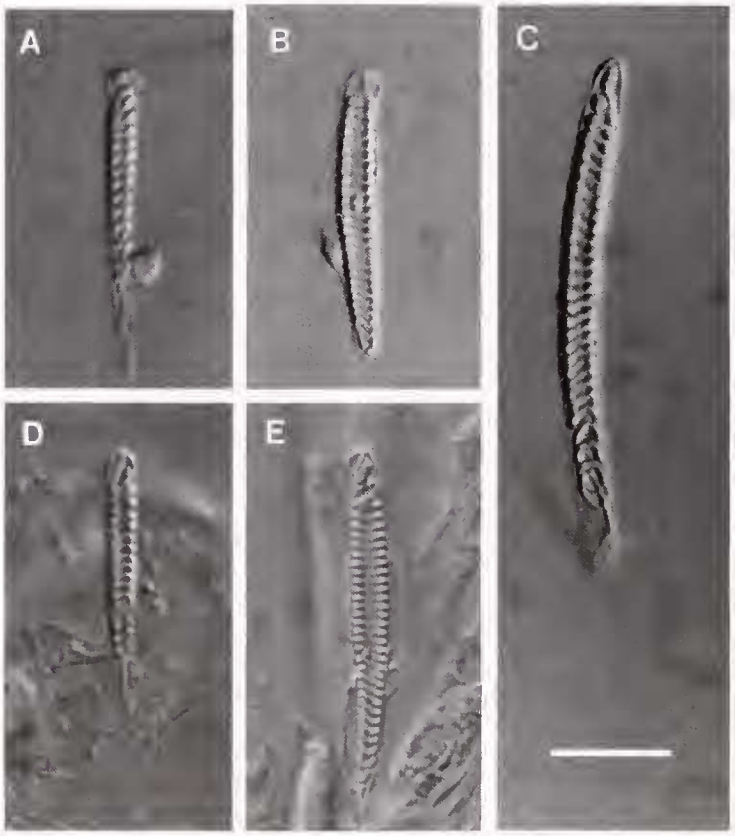


Figure 1. Comparison of average-sized spirocysts from three anemone species for specimens of the same size. In the living animals, the tubule end of the unfired spirocyst capsule (pointing upward in these photographs) presses tightly against the external cell membrane, so that the everting tubule is directed outward and away from the anemone during firing. Tentacle spirocysts are from *Anthopleura xanthogrammica* (**A**), *Anthopleura elegantissima* (**B**), and *Tealia crassicornis* (**C**). Acrorhagial spirocysts are from the same specimens of *A. xanthogrammica* (**D**) and *A. elegantissima* (**E**). Photographs were taken using differential interference contrast at 1000× (oil immersion). Each anemone weighed approximately 10 g. Scale bar 10 μm.

collected individuals of *Anthopleura xanthogrammica* and *Tealia crassicornis* (another sympatric, aclonal species). Specimens of *A. xanthogrammica* were selected to span the size range from within a single population that included very small juveniles. Specimens of *T. crassicornis* included freshly collected individuals from Anacortes, Washington (Shannon Point Marine Center [SPMC]) and a few very large individuals that had been kept in the laboratory for some years (at SPMC, Anacortes, WA, and the University of Washington Laboratories, Friday Harbor, WA).

Tissue selection

One medium-to-large tentacle was taken from each individual anemone. To reduce variability (Robson, 1988), I consistently examined only the proximal one-third of the largest tentacle for the size-graded series of anemones from a single clone. Acrorhagial tissue from the *Anthopleura* species was obtained from the white tip that contains specialized cnidae used only in aggressive interactions with other anthozoans.

Spirocyst identification and measurement

Unfired spirocysts are easy to recognize in squash preparations of either fresh or preserved tissue. The capsule wall is unusually transparent, and the crystalline material inside the inverted tubule produces very strong birefringence. Under interference contrast illumination, spirocysts look like glowing tapered springs that have been squashed slightly in their transparent capsule sacks (Fig. 1). I prepared smears by macerating a sample of tissue mechanically in a drop of seawater and pressing this suspension into a thin layer between a slide and coverslip. Spirocysts were identified using a light microscope at 1000× (oil immersion) and measured to the nearest 0.1 μm using a computer-linked video camera (image analysis) system. Data on spirocyst sizes were collected systematically by scanning each slide

(as described in Williams, 1996) and measuring maximum lengths and widths (method of Hand, 1954) of the first 20 clearly visible, intact, and unfired capsules with their long axes parallel to the plane of the slide. Duplicated measurements using 20 video images indicated that spirocyst dimensions were reproducible within ± 1% (lengths) and ± 4% (widths).

Data analyses

For these data, no single refined and appropriate statistic exists for description and hypothesis testing, so different analyses were used for different purposes. Here I apply a series of generally conservative and robust statistics to describe and compare data on cnida size as a function of polyp size.

Mean capsule dimensions and their standard errors were calculated for systematic samples of 20 measurements from each tissue, which provided a good estimate of the true means (Williams, 1998). Subsequent analysis of variation in these means, rather than the raw data, offers better resolution for detecting real, between-sample differences, especially where levels of within-sample variability are very high, as they are here.

The nonparametric Spearman rank sum correlation (one-tailed test) was used to determine *P*-values for correlations between mean capsule dimensions and polyp wet weights (Zar, 1984), with groupwise error (asterisks) determined using the sequential Bonferroni adjustment (Jaccard and Wan, 1996).

Graphs were constructed using a linear scale on the *Y*-axis and log scale (for wet weight) on the *X*-axis (Longley, 1984) to eliminate the perceptual bias of the traditional log-log plot typically used in scaling studies (Smith, 1984). To compute scaling exponents, anemone wet weights and mean dimensions of the spirocyst capsules were transformed to common logarithms (base 10) for analysis.

Model II regression—slopes (scaling exponents) and intercepts for log-log plots. Since specimens were deliberately selected to span the existing size range of the anemones, the wet weights (and their logs) are not normally distributed. Furthermore, all of the variables are measured with error; and measurement error for the *X* and *Y* variables is similar (± 3% for wet weights and ±1%–4% for cnida dimensions). Thus these data do not meet the assumptions for Model I regression analysis (McArdle, 1988), nor for associated parametric methods such as ANOVA, ANCOVA, and multivariate analysis. For *R* values less than 0.9, the ordinary least squares (OLS) method typically underestimates the scaling exponent; and for R greater than 0.9, predictions of the Model I and Model II regressions converge (McArdle, 1988; LaBarbera, 1989).

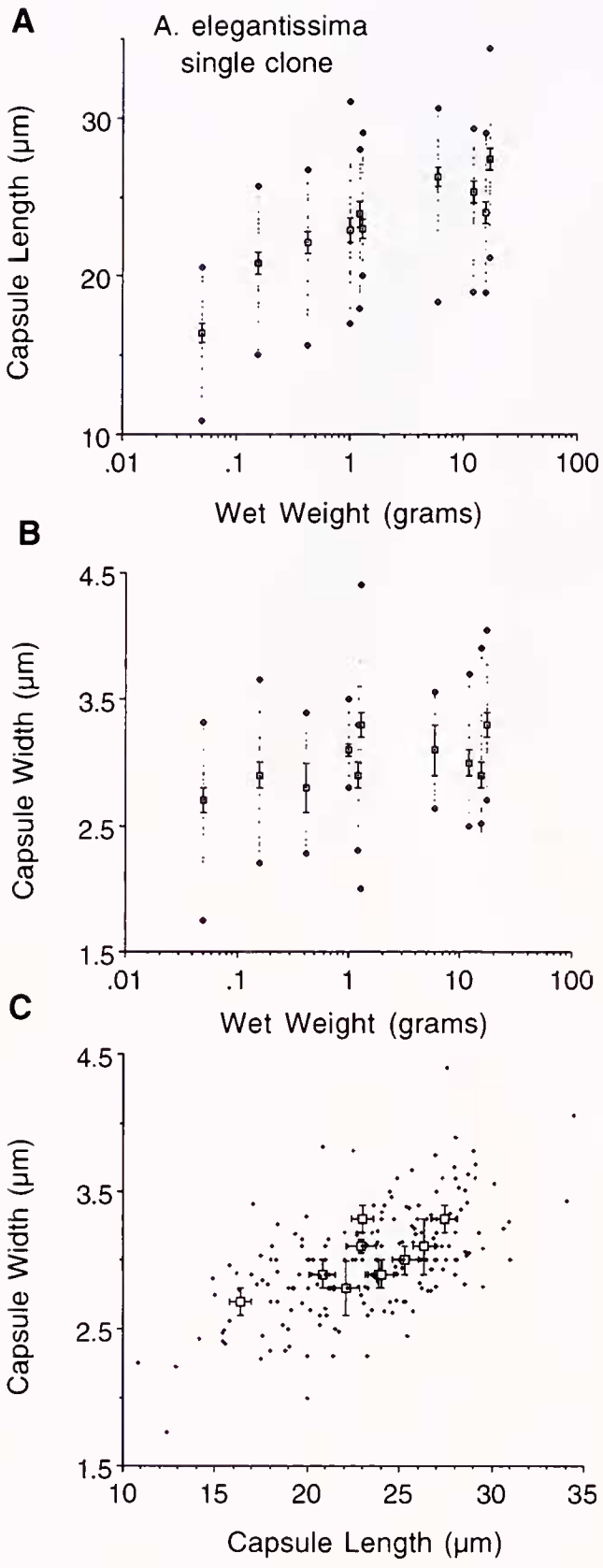
Model II regression is appropriate for describing "functional relationships" such as these (Sokal and Rohlf, 1981; Rayner, 1985). Hence, to determine scaling exponents (slope of the log-log plot), lines were fitted to the log-transformed data using the reduced major axis (RMA) method, also known as the geometric mean regression, probably the most robust method for determining the slope for morphometric data of this kind (McArdle, 1988; LaBarbera, 1989). In addition to being scale-independent, the RMA estimate of the slope (b_{RMA}) is rotation-invariant (Smith, 1984; Rayner, 1985) and easily calculated from the OLS estimate of the slope (b_{OLS}) and *R*, the Pearson product moment correlation ($b_{RMA} = b_{OLS}/R$). The asymmetrical 95% confidence intervals (95% CIs) for the slopes were estimated using bootstrapping (10,000 iterations; SYSTAT, 1998, version 9; SPSS Inc., Evanston, IL). None of the conclusions in this study were sensitive to the choice of regression models.

Goodness of fit and standard error of estimate (SEE). The Pearson product moment correlation (*R*) is affected by the slope of the line and the range of values of both variables, and thus is a biased measure of the goodness of fit (Smith, 1984). Since *R* decreases as the slope of the line decreases, this is particularly problematic where the slopes are quite small, as they are here. As a measure of the strength of association, Smith (1984) recommended reporting the standard error of estimate (the standard deviation of residuals which is sometimes called "standard error of regression"), expressed as a percent of the mean *Y* value, for ease of interpretation. For log data, percent standard error of estimate can be calculated as follows:

$$\%\text{SEE} = \text{Antilog}(2 + \text{SEE as a log}) - 100$$

(Smith, 1984). Because the variances of RMA and OLS estimators are identical to the third significant digit (McArdle, 1988), I report *R* and SEE values based on OLS regression calculations.

Significance of between-sample differences. Comparisons between tissues and species were *a priori* tests of initial hypotheses, and were based on paired samples from the same individuals or from weight-paired individuals of different species. Paired student's *t*-tests (Sokal and Rohlf, 1981) were used to test for significant differences in the dimensions of tentacle and acrorhagial spirocysts from the same specimens. Between-species differences in capsule size were tested similarly by pairing polyps by weight (Zar, 1984) and testing for significant differences in spirocyst capsule dimensions and in polyp wet weights (weight differences never significant, here). The paired *t*-test assumes only that the differences (and not the variables themselves) are normally distributed (Zar, 1984). This method provides several advantages over simply comparing *Y*-intercepts us-



ing estimated 95% confidence intervals. (1) Conclusions are based on direct comparison of the data and are thus independent of assumptions used in calculating the best fit line and its confidence intervals. (2) Assessment is based on consistency across the overlapping range of body sizes, rather than on predicted differences for one arbitrary body size. (3) Providing *P*-values allows correction for groupwise error.

Scaling exponents were considered to be significantly different if one lay outside the 95% confidence interval for the other. Where scaling exponents do not differ significantly, line elevations may be compared similarly using the 95% CIs for the *Y*-intercepts (Hess, 1993).

Results

Average capsule length of the tentacle spirocysts increased continuously and significantly with increasing polyp size in all three macrophagous anemone species (Figs. 2A–5A; Table 1). Over the size ranges sampled, capsule lengths increased 22%–67%. Smaller increases in the capsule widths (0%–22%) generally were not significant at these sample sizes (Figs. 3B–5B, Table 2). The same patterns were apparent within a single clone of *Anthopleura elegantissima* (Fig. 2) where among-sample variation was minimized, and the increase in capsule width reached significance.

Scaling of tentacle spirocysts

Although the size of the tentacle spirocysts increased with body size in all cases, the spirocysts exhibited strong negative allometry. That is, scaling exponents for mean capsule length (0.052–0.086) and mean capsule width (0.021–0.039) were significantly smaller than the 0.33 predicted for proportional growth (= isometry for dimension length as a function of dimension mass), and smaller than reported scaling exponents for anemone basal diameters (0.30–0.38; Sebens, 1981a) (95% confidence intervals; Tables 1, 2).

Scaling of acrorhagial spirocysts from Anthopleura

For the genetically diverse sample of *A. elegantissima*, both capsule width and capsule length of the acrorhagial spirocysts increased significantly with increasing body

Figure 2. Increase in the size and aspect ratio of tentacle spirocysts as a function of body size within a single clone of the sea anemone *Anthopleura elegantissima*. Data are mean capsule dimensions (open squares), plus or minus their standard errors (bars), for 20 undischarged tentacle spirocysts (dots or small crosses) from each of 10 individuals from this clone. Polyp ranges are shown as open diamonds. Mean capsule length (**A**) and width (**B**) are shown as functions of anemone wet weight. Widths of the individual capsules (**C**) are shown as a function of individual capsule length for the pooled sample of 200 spirocysts from these 10 anemones.

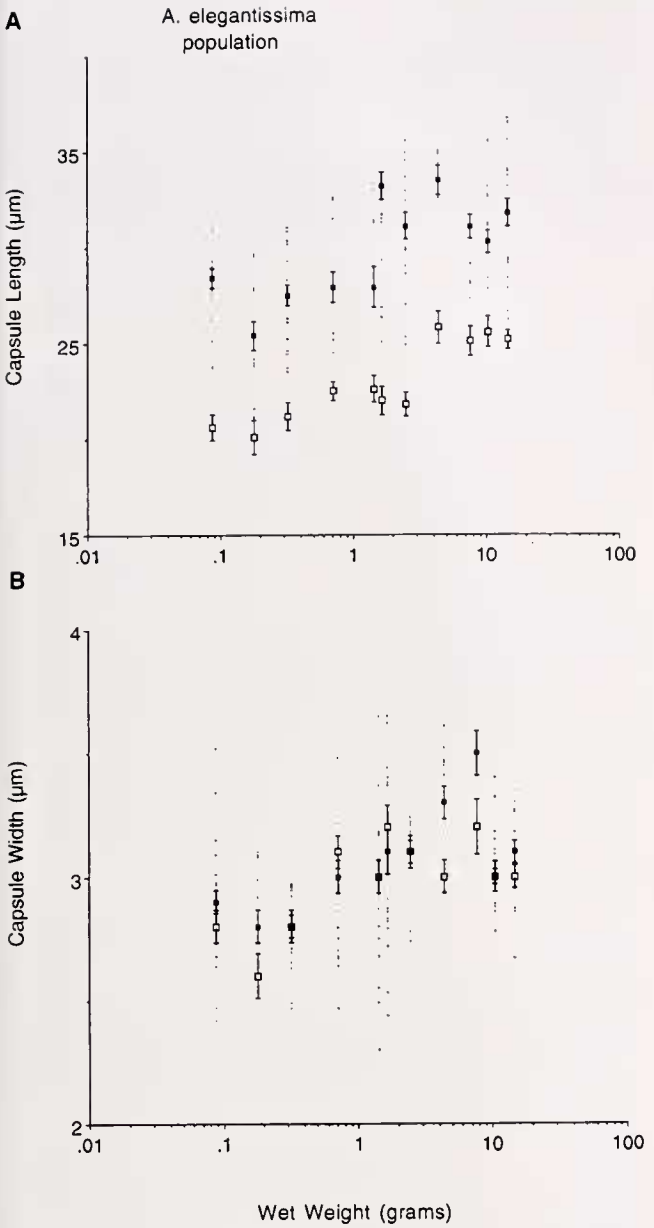


Figure 3. Length and width of acrorhagial spirocysts (solid squares) and tentacle spirocysts (open squares) from a genetically diverse sample of the clonal anemone *Anthopleura elegantissima*. Data are means, plus or minus their standard errors (bars), for 20 undischarged spirocysts (dots for tentacle spirocysts only) from each of the two tissue samples taken from individuals with well-developed acrorhagi (n = 11 individuals from 8 different clones). Average capsule lengths (**A**) and widths (**B**) are shown as functions of body size, for tentacle spirocysts and acrorhagial spirocysts from the same anemones.

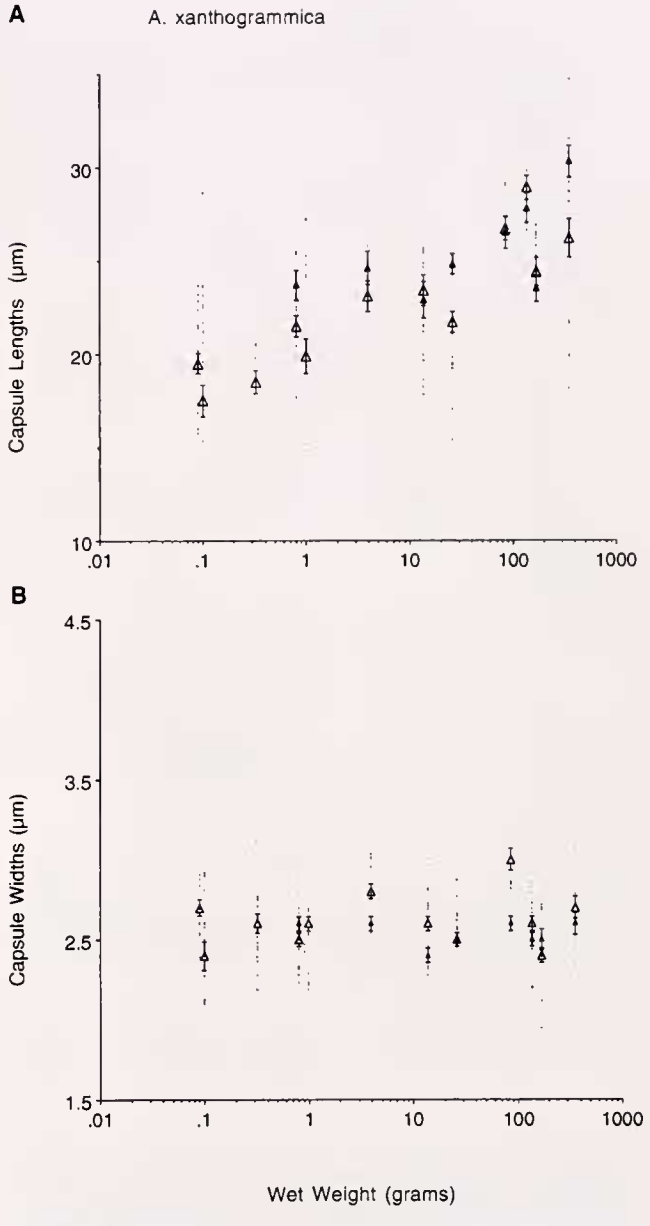


Figure 4. Length and width of acrorhagial spirocysts (solid triangles) and tentacle spirocysts (open triangles) of the aclonal anemone *Anthopleura xanthogrammica*. Data are means, plus or minus their standard errors (bars), for 20 undischarged spirocysts (dots for acrorhagial spirocysts only) from each of the two tissue samples taken from the same individuals. Because specimens of *A. xanthogrammica* smaller than 0.8 g wet weight consistently lack acrorhagi, there are more samples of tentacle spirocysts (n = 12 polyps) than of acrorhagial spirocysts (n = 8 polyps). Average capsule length (**A**) and width (**B**) are shown as functions of body size, for tentacle spirocysts and acrorhagial spirocysts from the same anemones.

weight (Tables 1, 2; Fig. 3). In contrast, for the non-clonal anemone *A. xanthogrammica,* neither width nor length of the acrorhagial spirocysts was significantly correlated with body size (Tables 1, 2). However, the consistent absence of acrorhagi in very small individuals reduced the size range of this sample (n = 8 individuals with acrorhagi; Fig. 4).

Variation in capsule shape

In all six spirocyst populations (three from *A. elegantissima,* two from *A. xanthogrammica,* and one from *Tealia crassicornis*), scaling exponents for capsule width (Table 2)

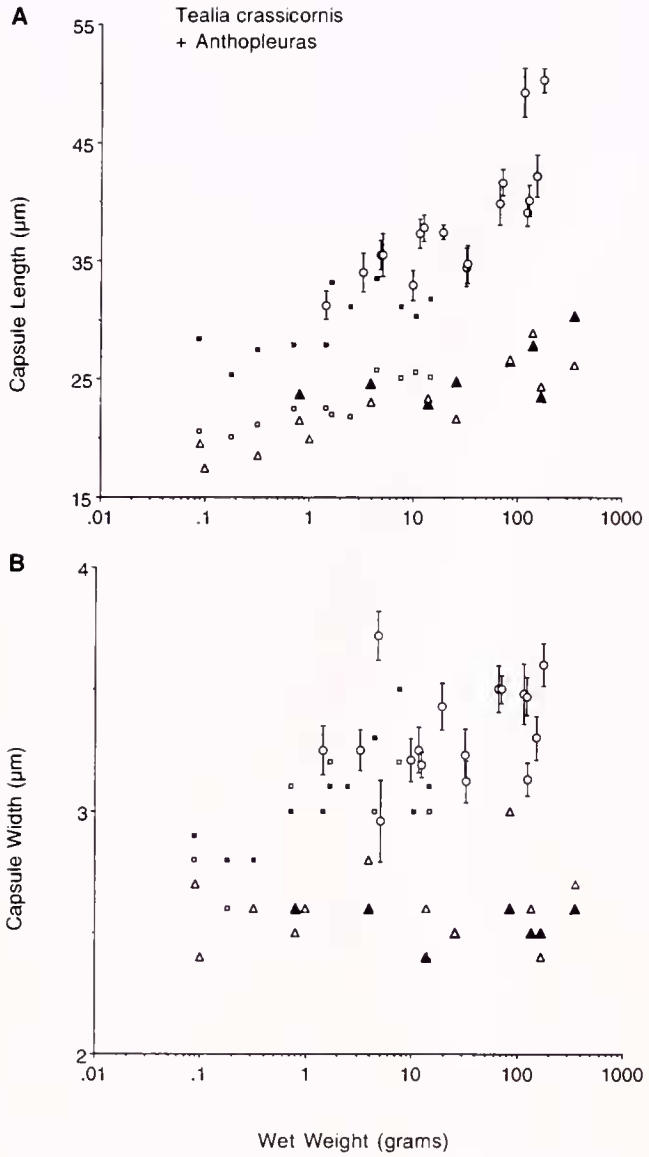


Figure 5. Scaling of tentacle spirocysts from *Tealia crassicornis* (circles), compared with scaling of tentacle and acrorhagial spirocysts from *Anthopleura elegantissima* (squares) and *A. xanthogrammica* (triangles). New data for *T. crassicornis* are means, plus or minus their standard errors (bars), for 20 spirocyst capsules from each sample. Average capsule length (**A**) and capsule width (**B**) are shown as functions of body size for tentacle spirocysts from *T. crassicornis* (means and their standard errors), with comparative data for tentacle spirocysts (open symbols) and acrorhagial spirocysts (filled symbols) from *A. xanthogrammica* and the genetically diverse sample of *A. elegantissima*. For clarity, the data for the two *Anthopleura* species from previous figures (Figs. 3, 4), are shown here without error bars.

were smaller than those for capsule length (Table 1). Except for the *A. elegantissima* acrorhagi, these differences were all significant statistically (*i.e.*, predicted exponents for capsule width were outside the 95% confidence intervals for capsule length exponents). Thus capsule aspect ratios increased consistently with capsule size in both tissues and for all three anemone species.

This shape variation was also apparent within the *A. elegantissima* clone: the scaling exponent for log individual capsule width as a function of log capsule length (Table 2; Fig. 2C) was 0.66, which was significantly smaller than the theoretical value of 1.0 predicted for proportional (isometric) scaling (95% confidence intervals; Table 2).

Differences in scaling exponents between tissues and species

Scaling exponents for capsule length did not differ significantly between the two *Anthopleura* species, for either tentacle or acrorhagial spirocysts (slopes all lay within each others' 95% confidence intervals, Table 1). Although the tentacle spirocysts from *T. crassicornis* showed a slightly higher scaling exponent than tentacle spirocysts from the genetically diverse samples of *A. elegantissima* and *A. xanthogrammica*, this exponent was not significantly higher than that of the *A. elegantissima* clone (Table 1). Furthermore, the higher scaling exponent of *T. crassicornis* was heavily influenced by two of the largest individuals (see Fig. 5A).

By contrast, the scaling exponents for capsule width were significantly lower for spirocysts from *A. xanthogrammica* tentacles and acrorhagi than for the other two anemones, whose capsule width exponents were indistinguishable from each other.

Spirocyst size: significant differences between tissues and species

Consistent differences in mean capsule size are clearly apparent from the combined semilog plot of polyp means (Fig. 5), and from photographs (Fig. 1) of average-sized spirocytes taken from specimens of the same size (10 g wet weight). Tentacle spirocysts from the genetically diverse sample of *A. elegantissima* were significantly shorter (paired *t*-test, $P < 0.001$*), but not narrower ($P < 0.2$), than their own acrorhagial spirocysts, and were both shorter and narrower than the tentacle spirocysts from *T. crassicornis* ($P < 0.05$*). Both tentacle and acrorhagial spirocysts from *A. xanthogrammica* were significantly shorter and narrower ($P < 0.05$*) than the intermediate-sized tentacle spirocysts of *A. elegantissima*.

Discussion

Cnida scaling is described here for the first time. Of the possible questions about this newly described phenomenon, here are a few that I find particularly compelling. Are cnida scaling and allometry real, characterizable, and distinctive kinds of intraspecific variability? Are such patterns rare, common or universal among cnidae? What effects might

Table 1

Spirocyst capsule length vs. anemone size: scaling exponents and constants for three anemone species, from reduced major axis regression equations

Species Tissue (*n*)§	Scaling exponent† (95% CI)	Intercept† (95% CI)	$\bar{X}$, $\bar{Y}$‡	*R*¶ (%SEE)	*P*#
Log mean spirocyst capsule length in μm (*Y*) *vs.* log anemone wet weight in grams (*X*) (see Figs. 2A–5A for data)					
Anthopleura elegantissima, single clone					
Tentacles (10 anemones)	0.072 (0.041–0.092)	22.4 (22.1–22.6)	0.1998, 1.362	0.897 (7.0%)	<0.0005***
A. elegantissima, genetically diverse sample					
Tentacles (11)	0.053 (0.043–0.072)	22.3 (22.1–22.5)	0.185, 1.359	0.920 (4.1%)	<0.0005**
Acrorhagi (11)	0.049 (0.033–0.079)	29.1 (28.7–29.3)	0.185, 1.474	0.791 (6.6%)	<0.025*
Anthopleura xanthogrammica					
Tentacles (12)	0.052 (0.039–0.065)	20.4 (19.9–20.8)	0.780, 1.349	0.908 (7.1%)	<0.0005**
Acrorhagi (8)	0.046 (0.022–0.091)	23.0 (18.6–23.6)	1.485, 1.405	0.632 (8.3%)	<0.05
Tealia crassicornis					
Tentacles (17)	0.086 (0.054–0.121)	28.8 (27.7–32.0)	1.402, 1.581	0.806 (8.3%)	<0.0005***

§ Number of anemones sampled (20 capsules per sample).

† Slope and detransformed *Y*-intercept (μm) with 95% confidence intervals.

‡ Mean values of (log *X*) and (log *Y*).

¶ Pearson's product-moment correlation coefficient (*R*) and percent standard error of estimate (%SEE).

P-value calculated for Spearman rank correlation with groupwise significance after sequential Bonferroni adjustment, where * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

cnida scaling and allometry have on the structure, function, development, and evolution of cnidae and anemones? And most intriguing to me, what *causes* cnida scaling? Proximally, how might gross body size influence the size and shape of these intracellular microtools; and ultimately, are these patterns likely to be advantageous?

Intraspecific variation in cnida size

First and most importantly, cnida scaling is real: larger *Anthopleura* and *Tealia* do produce larger spirocysts. Furthermore, a reexamination of published data suggests that cnida scaling is actually widespread among anemones (Table 3). Of the 39 studied populations, 25 show positive correlations between *cnida length* and polyp size, and 3 of 13 show positive correlations between *cnida width* and polyp size. Curiously, rather than characterizing and explaining between-sample variability, previous studies have focused exclusively on defining invariant characters for species description and phylogeny (Stephenson, 1929; Weill, 1934a, b; Carlgren, 1949; Williams, 1996, 1998, 2000; Chintiroglou, 1996; Chintiroglou *et al.*, 1996, 1997; Chintiroglou and Simsiridou, 1997; Chintiroglou and Karalis, 2000). In this search for stable taxonomic characters, cnida scaling and other examples of intraspecific variability have typically been dismissed as inconvenient or unworkable (but see Ryland *et al.*, 2004). Although cnida scaling appears widespread among anemones, I am not aware of comparable information for other cnidarians.

Second, cnida size varies continuously. No size classes exist within individual samples (Figs. 2–4), or within the pooled sample from a single clone (Fig. 2C).

Variation in cnida shape with increasing cnida size

Multiple lines of evidence indicate that cnida shape changes with cnida size. This is particularly apparent from the combined sample of 200 tentacle spirocysts from 10 clonemates of *A. elegantissima* (Fig. 2C, Table 2). The width of individual capsules did increase as a function of capsule length; but the scaling exponent of 0.66 was significantly less than the scaling exponent of 1.0 expected for isometry ($P < 0.001$). In all tissues, all species, and all cnida types examined, cnida length increased more dramatically with increasing anemone size than did capsule width; the single exception being the acontial b-mastigophores from *Sagartia elegans* (data from Stephenson, 1929; Kramer and Francis, 2004; this study, Fig. 5, Tables 1–3). Size-range data in species descriptions show the same pattern. For 39 distinctive cnida populations from *A. elegantissima*, *T. crassicornis*, and *A. xanthogrammica*, the average percent difference between reported minimum and

Table 2

Spirocyst capsule width vs. anemone size or capsule length: scaling factors and constants for three anemone species, from reduced major axis regression equations

Species Tissue (n)§	Scaling exponent† (95% CI)	Intercept† (95% CI)	$\bar{X}$, $\bar{Y}$‡	R¶ (%SEE)	P#
a) Log mean spirocyst capsule width in μm (Y) vs. log anemone wet weight in grams (X) (see Figs. 2B–5B for data)					
Anthopleura elegantissima, single clone					
Tentacles (n_A = 10 anemones)	0.032 (0.021–0.048)	2.96 (2.93–2.976)	0.1998, 0.476	0.587 (5.9%)	<0.05*
A. elegantissima, genetically diverse sample					
Tentacles (n_A = 11)	0.036 (0.022–0.056)	2.94 (2.91–2.948)	0.185, 0.474	0.780 (5.1%)	<0.1
Acrorhagi (n_A = 11)	0.038 (0.020–0.061)	3.00 (2.97–3.02)	0.185, 0.484	0.783 (5.1%)	<0.01*
Anthopleura xanthogrammica					
Tentacles (n_A = 12)	0.021 (0.012–0.033)	2.51 (2.46–2.56)	0.780, 0.417	0.202 (6.8%)	>0.5
Acrorhagi (n_A = 8)	0.014 (0.007–0.028)	2.42 (2.31–2.48)	1.485, 0.404	0.126 (3.2%)	>0.5
Tealia crassicornis					
Tentacles (n_A = 17)	0.039 (0.025–0.056)	2.93 (2.77–3.07)	1.402, 0.521	0.312 (6.0%)	<0.25
b) Log individual capsule width in μm (Y) vs. log individual capsule length in μm (X) (see Figs. 2C for data)					
A. elegantissima, single clone					
Tentacles (n_C = 200 capsules)	0.661 (0.583–0.746)	0.375 (0.287–0.48)	1.3601, 0.474	0.60 (10.9%)	<0.0005**

§ (n_A) = number of anemones sampled (20 capsules per sample); (n_C) = number of individual spirocyst capsules.

† Slope and detransformed Y-intercept (μm) with 95% confidence intervals.

‡ Mean values of (log X) and (log Y).

¶ Pearson's product-moment correlation coefficient (R) and percent standard error of estimate (%SEE).

P-value calculated for Spearman rank correlation with groupwise significance after sequential Bonferroni adjustment, where * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

maximum capsule dimensions was 56% for capsule widths and 102% for capsule lengths (data from Hand, 1955). Larger cnidae typically become disproportionately longer, regardless of cnida type, tissue, or species.

Taxonomic implications of intraspecific variation in cnida size and shape

Because cnida size and shape commonly change with body size within anemone species, their value as taxonomic characters is much reduced (Williams, 1996, 1998, 2000). Although taxonomists have acknowledged that intraspecific variability can be problematic (review by Fautin, 1988), cnida size ranges have typically been used in species descriptions, rather than mean cnida dimensions (± SD) for each specimen, because this saves time and space. I strongly recommend including specimen wet weights in all future work, and wherever possible, also including mean capsule dimensions (± SD) for a 1-g specimen, as a basis for quantitative comparisons between populations and species. For more detailed comparative work, cnida scaling exponents and the Y-intercepts (predicted capsule dimensions for a 1-g specimen) provide distinctive characters that may be particularly valuable for separating cryptic species (*e.g.*, *Metridium senile* and *M. farcimen*, Kramer and Francis, 2004).

Evolutionary significance of larger acrorhagial spirocysts in A. elegantissima

Increasing localization and specialization of cnidae is a common evolutionary trend among sea anemones (Schmidt, 1974). Specialization through enlargement of the acrorhagial spirocysts in *A. elegantissima* (this study) and its sibling species, *A. sola* (Pearse and Francis, 2000), appears to be an example of this pattern. Acrorhagial and tentacle spirocysts are the same size (and relatively small) in *A. xanthogrammica* (this study) and also in the more distantly related *A. artemisia* (Hand, 1965), which belongs to a different branch of the genus (Geller and Walton, 2001). Larger acrorhagial spirocysts may be a shared derived character linking the sibling species pair *A. elegantissima* (this study) and *A. sola*, and distinguishing them from the closely related *A. xanthogrammica*.

Table 3

Correlation of cnida dimensions with body size for 39 cnida populations from 9 anemone species

Anemone species	Sample size§ $n \times N$	Correlated†		Data source
		Capsule length	Capsule width	
Anthopleura elegantissima	20 × 11	2/2	1/2	Francis, this study
Anthopleura xanthogrammica	20 × 12	1/1	0/1	Francis, this study
	20 × 08	0/1	0/1	
Actinia equina mediterranea				
form I	40 × 20	6/7	—	Chintiroglou and Simsiridou, 1997
form II	40 × 20	3/7	—	Chintiroglou *et al.*, 1997
Cereus pedunculatus	30 × 07	1/2	1/2	Stephenson, 1929 (calculations here)
Edwardsia claparedii	40 × 10	4/8	—	Chintiroglou, 1996
Metridium senile	20 × 08	4/4	0/2	Kramer and Francis, 2004
Metridium farcimen	20 × 10	1/2	—	Kramer and Francis, 2004
	20 × 21	1/1	0/1	
	20 × 27	1/1	1/1	
Sagartia elegans	30 × 12	0/2	0/2	Stephenson, 1929 (calculations here)
Tealia crassicornis	20 × 17	1/1	0/1	Francis, this study‡
Totals	5120 capsules	25/39 correlated	3/13 correlated	

§ Number of cnidae per sample (n) times the number of polyps sampled (N).

† Correlated capsule lengths and capsule widths = proportion of cnida populations with significant positive correlation between capsule dimension and polyp size (*e.g.*, 5/8 = 5 correlated and 3 not significantly correlated).

‡ With assistance from Paul Mages.

Functional significance of cnida size and shape

Within the functional limits of a given design, cnida effectiveness may commonly increase with cnida size (see Purcell, 1984). The cnidae are extraordinarily complex collagenous, intracellular secretions serving a wide range of general and specialized functions. They are numerous, costly, and discarded after a single use, puncturing the enclosing cell in the process. Thus their production and use must entail considerable investment by the animals. Larger capsules can carry larger payloads of tubules, spines, barbs, venoms, or glues. Furthermore, fewer cnidocytes are sacrificed during the firing of a few large spirocytes than by firing many small ones. Consequently larger cnidae probably contribute to other advantages of large body size in anemones, including advantage in competition, escape in size from predation, and ability to handle larger prey (Francis, 1979; Sebens, 1983, 1987; Kramer and Francis, 2004).

Variation in cnida shape may also impact function. Based on mechanical analysis of pressure vessel geometry, larger cnidae may typically become more elongate, rather than stouter, because of the impact of capsule width on wall stress. Local tension in the walls of a pressurized cylinder increases in proportion to the diameter and is unaffected by the length (Alexander, 1983, p. 153). For the cnidae, this means that wider capsules with the same internal pressure will experience more distortion in the walls, and presumably also more distortion of the catch/latch mechanism, and therefore greater risk of wasteful spontaneous firing. Increasing cnida size beyond the point of spontaneous firing would require a change in cnida design (*e.g.*, thicker cnida walls or a different latch design). This may be one reason that capsule widths increase relatively slowly as cnida size increases with anemone size.

The more elongate shape of larger cnidae may also be important for rapid influx of water during cnida firing (Thomason, 1988). Increase in cnida size without shape change (capsule isometry) would reduce the surface-to-volume ratio (S/V), causing more delayed firing in larger cnidae. The short, narrow spirocysts of *A. xanthogrammica* should be the fastest in this group, both because of their relatively high S/V ratios and because the relatively shorter tubule in these small spirocysts (Fig. 1A, D) should complete eversion more quickly than the longer tubules in the larger spirocysts of *A. elegantissima* and *T. crassicornis* (Fig. 1B, C, E).

Adaptive significance of cnida size, shape, and scaling

Differences in cnida size are likely to be adaptive. For example, relatively higher investment in competitive interference was predicted for clonal than for non-clonal anemones (Francis, 1988), which is reflected in the relatively larger acrorhagial spirocysts of *A. elegantissima* (this study). As another example, larger defensive nematocysts in the acontia of *Metridium farcimen* reflect the relatively higher predator densities in its subtidal habitat, by comparison with the smaller acontial nematocysts associated with the shallower habitat of *M. senile* (Kramer and Francis, 2004).

Differences in cnida shape (Fig. 1) are also likely to be

advantageous. For example, having short, narrow tentacle spirocysts that complete their firing very rapidly may be particularly advantageous for *A. xanthogrammica* (Fig. 1A), which captures loose mussels in wave-exposed areas (Sebens, 1981a, 1983) of high velocities and rapid accelerations (Denny *et al.,* 1985). A more delayed response by the wider and longer tentacle spirocysts of *A. elegantissima* (Fig. 1B) may be adequate for capturing smaller, lighter prey (Sebens, 1981a), and should be more economical in terms of cell (spirocyte) loss during firing. On the lower shore, *T. crassicornis* has very long and wide tentacle spirocysts (Fig. 1C) whose higher payload volumes are perhaps more important than a very rapid response time for capturing and holding crabs (Sebens and Laakso, 1978), which although powerful, are not fast-moving.

Cnida scaling may also be adaptive. For example, if larger spirocysts with their larger payloads permit greater adhesion to prey, then larger tentacle cnidae may yield increased capture success with larger prey. Both cnida size (this study) and prey size increase with body size for *A. xanthogrammica* (Sebens, 1981a) and for *A. elegantissima* (Spearman rank correlation for anemone size class *vs.* average prey size, two-tailed test, $r_s = 0.762$, $P < 0.05$; calculated using data from Sebens, 1981a). This connection between prey and cnida scaling is also supported by contrasting data for two planktivorous *Metridium* species, where prey size does not increase with body size (Sebens, 1981a). For *M. farcimen* and *M. senile,* the length of tentacle cnidae increases more slowly with body size (scaling exponents, 0.008–0.03, Kramer and Francis, 2004) than for macrophagous *Anthopleura* and *Tealia* species (scaling exponents, 0.052–0.086, this study). Thus cnida scaling patterns can be treated as features of cnidarian life histories.

Structural implications of cnida size and shape

From a design standpoint, providing space and support for needle-like cnidae and assuring the stability of surrounding soft tissues could be problematic, especially during cnida firing. Anemones have the greatest variety of cnidae in the class Anthozoa (Schmidt, 1974), which differs from Scyphozoa and Hydrozoa in having elongate, rather than ovoid or spherical, cnidae (Mariscal, 1984). Like the cnidae that they support, the epithelial cells in anemones are unusually tall and thin, secreting and supported by an unusually fibrous and structured mesoglea which almost qualifies as connective tissue (Hyman, 1940; Chapman, 1966; Gosline, 1971; Koehl, 1977).

At the outer limits of stability, very dense arrays of very long cnidae in defensive structures (*e.g.,* capsule lengths ≅ 90 μm for *Metridium* acontial amastigophores and *Anthopleura* acrorhagial holotrichs; Hand, 1955) actually *do* cause local tissue disintegration during firing (Äbel, 1954; Francis, 1973b, Kramer and Francis, 2004). Maximum size of these cnidae may be limited by tissue thickness, or by any tendency to disrupt tissues during regular cycles of extreme body elongation and contraction (illustrations in Shick, 1991).

In contrast, cnidae in the feeding tentacles are less densely arrayed and smaller (capsule lengths ≅ 20–30 μm for *Tealia* and *Anthopleura;* Hand, 1955), and are not likely to disrupt the tissue during ordinary body movements. However, since tentacle cnidae are used frequently in numerous prey capture events, any tissue disruption during cnida firing will interfere with further prey capture, thereby favoring tissue stability in the feeding tentacles also. In both situations, cnida size is likely to be constrained more narrowly in smaller specimens with thinner tissues and less structural support.

Possible causes of cnida scaling

Cnida scaling could arise through natural selection where size-limiting factors apply more strongly to small animals. Limiting factors that may become progressively less restrictive with increasing body size include physiological and ecological factors such as the food and energy flux (Sebens, 1981a), as well as morphological factors such as tissue and mesoglea strength and stability (Shick, 1991), and physical limitations of space such as tissue thickness (Shick, 1991) and cell size (Peters, 1983; Calder, 1984; Stevenson *et al.,* 1995).

Since the thickness of both the mesoglea and the inner and outer epithelial layers of the anemone body typically does increase with body size (Shick, 1991), larger anemones should be able to accommodate and support larger cnidae. While this may explain scaling of exceptionally long cnidae that are at or near the limits of tissue tolerance (*e.g.,* capsule length of acrorhagial holotrichs ≅ 90 μm; Hand, 1955), it would not explain scaling of the much smaller acrorhagial spirocysts interspersed among them (capsule lengths ≅ 30μ, this study). Clearly, then, not all instances of cnida scaling are due to gradual release of the more rigorous spatial and support constraints on smaller individuals.

In fact, no one of these factors can explain all the available data. If increase in cnida size were purely a function of higher energy availability in larger specimens, then cnida size should increase similarly with body size in all the tissues of a species. In fact, scaling exponents for the tentacle cnidae are typically significantly smaller than for the acontial cnidae in both *Metridium senile* and *M. farcimen* (Kramer and Francis, 2004). Since scaling exponents may vary between tissues as well as between species, cnida size clearly is not controlled uniformly by size-related differences in the energy budget of the whole animal.

Cnida scaling also occurs where there is no obvious selective advantage based on functional differences between larger and smaller individuals. Although prey size *does not*

increase with body size for *Metridium* (Sebens, 1981a), larger individuals *do* have longer tentacle cnidae (Kramer and Francis, 2004), although these scaling exponents are unusually small (0.018–0.03, as compared with 0.052–0.072 for *Anthopleura* tentacle spirocysts, and 0.035–0.051 for *Metridium* acontial nematocysts). Thus larger tentacle cnidae in larger individuals cannot always be explained as aiding in the capture of larger prey.

Developmental significance of cnida size differences

Cnida scaling may be caused proximally by cell scaling. In *Hydra*, cnida size is related to cell size, which declines with the number of divisions that an interstitial cell undergoes before differentiation. More divisions result in more and smaller cnidocytes, which produce smaller cnidae (Lehn, 1951). This may also be true for anemones, where otherwise continuous cnida populations often include an occasional double-sized or half-sized capsule (Cutress, 1955; Daphne Fautin, University of Kansas, pers. comm.), perhaps produced by one division more or less than the usual for that interstitial cell line.

A direct relationship between cell size and the size of secreted structures may actually be rather common. In *Drosophila*, local polyploidy in bristle-secreting cells results in larger cells that secrete larger bristles (Adler *et al.*, 2000); and in the roundworm *Caenorhabditis elegans*, a mutation that produces adult dwarfing has been traced to miniaturization of a cell line that contributes to the size of the cuticle-secreting and syncytial hypodermis (Knight *et al.*, 2002).

If cell size determines cnida size, then cnida scaling presumably implies cell scaling. That is, the observation that larger anemones have larger cnidae implies that the size of the cnidocytes also varies continuously, and reversibly, as a function of anemone body size.

Implications for cell and organelle scaling

While growth typically involves changes in body proportions, the fact that this also occurs at the cellular and subcellular level may be surprising (Brown and West, 2000). Order-of-magnitude agreement between cnida scaling exponents (0.018–0.086; this study; Stephenson, 1929; Kramer and Francis, 2004) and cell scaling exponents (0.017–0.17; Peters, 1983; Calder, 1984; Stevenson *et al.*, 1995) suggests that cnida scaling may reflect a wider pattern of cell scaling.

Based on these accurate cnida measurements, a power law for cnidae and cells may be closer to 1/10 than to the well-known 2/3–3/4 power law for gross morphological and physiological functions. Measured scaling exponents for the lengths (b_L = 0.052–0.086) and widths (b_W = 0.021–0.039) of unfired cnidae can be used to estimate scaling exponents for cnida surface areas ($b_{SA} = b_L + b_W$ = 0.073–0.125) and volumes ($b_V = b_L + 2b_W$ = 0.094–0.164) as functions of body mass. Thus cnida functions that depend on capsule surface area or volume should have exponents between 0.073 and 0.164, quite different from the 0.67–0.75 exponents reported for gross body functions (Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Niklas, 1994).

As a general explanation for the scaling of epithelial secretory cells and their structural products (such as extracellular mesoglea and basement membrane), I suggest that larger, taller epithelial cells can supply more structural material per unit attachment area for building and maintaining thicker sheets of pliant, extracellular support materials to resist the increased wall stresses in larger, stouter pressurized cylinders of all kinds—including worms, blood vessels, and guts, as well as sea anemones (Francis, unpubl. obs.). The scaling of cnidocytes and their intracellular cnidae can be considered a special, restricted case of this more general phenomenon, since cnidae cannot be larger than the cells enclosing them.

Acknowledgments

Data for *Tealia crassicornis* were collected with Paul Mages with support of REU funding (NSF 0097190). Andrew Kramer wrote the algorithm and ran the bootstrapping program. For able assistance, I thank Antonia Crease, students Deanna Wynn, Cliff Hollins, and Paul Mages (NSF 9729816 and NSF9731144), and volunteers Deborah S. Frazee, Margaret Longley, Misa Peterman, Carley Reiter, Keith Tuley, and Richard Wills; for office space, Anne H. Pope; for helpful discussion and editing, Michael LaBarbera, A. Richard Palmer, Alison and Roger Longley, and Garry Odell; for access to facilities, the directors, staff, and colleagues at Shannon Point Marine Center (Western Washington Univ.), Long Marine Laboratory (Univ. of California, Santa Cruz), and Oregon Institute of Marine Biology (Univ. of Oregon); and for the image analysis system at Shannon Point, the NSF Instrumentation and Laboratory Improvement Program and Texaco Foundation. The anemones themselves and the beautiful work of T. A. Stephenson provided wonderful inspiration. Without such support, the work would not have been possible.

Literature Cited

Äbel, E. F. 1954. Ein Beitrag zur Giftwirking der Actinien und Function der Randsäckchen. *Zool. Anz.* **153:** 259–268.

Acuña, F. H., and M. O. Zamponi. 1997. The use of cnidocysts for ecological races identification from sea anemones populations (Anthozoa, Actiniidae). *Iheringia Ser. Zool.* **82:** 9–18.

Adler, P. N., J. C. Liu, and J. Charlton. 2000. Cell size and the morphogenesis of wing hairs in *Drosophila*. *Genesis* **28:** 82–91.

Alexander, R. McN. 1983. *Animal Mechanics*, 2nd ed. Blackwell Scientific, Oxford.

Blanquet, R. S. 1988. The chemistry of cnidae. Pp. 407–425 in *The*

Biology of Nematocysts, D. A. Hessinger and H. Lenhoff, eds. Academic Press, San Diego, CA.

Brown, J. H., and G. B. West, eds. 2000. *Scaling in Biology*. Oxford Univ. Press, New York.

Calder, W. A. III. 1984. *Size, Function, and Life History*. Harvard Univ. Press, Cambridge, MA.

Carlgren, O. 1949. A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *K. Sven. Vetenskapsakad. Handl.* **1:** 1–121.

Chapman, G. 1966. The structure and functions of the mesoglea. Pp. 147–168 in *The Cnidaria and Their Evolution*, W. J. Rees, ed. *Symp. Zool. Soc. Lond.* No. 16.

Chintiroglou, C. C. 1996. Biometric study of *Edwardsia claparedii* (Panceri) cnidome (Actiniaria: Anthozoa). *Belg. J. Zool.* **126:** 177–180.

Chintiroglou, C. C., and P. Karalis. 2000. Biometric investigations on the cnidae of the Aegean colour morphs of *Anemonia viridis*. *J. Mar. Biol. Assoc. UK* **80:** 543–544.

Chintiroglou, C. C., and M. Simsiridou. 1997. Biometric investigations on the cnidae of the sea anemone *Actinia equina mediterranea* Form 1 *sensu* Schmidt, 1972. *Isr. J. Zool.* **43:** 5–11.

Chintiroglou, C. C., D. Doumenc, J. Le Renard, A. Fouhert, and Ph. Kolyva-Machaira. 1996. Classification of cnidarian nematocysts using multivariate and digital image analysis. *Bios (Macedonia, Greece)* **4:** 123–135.

Chintiroglou, C. C., I. Christou, and M. Simsiridou. 1997. Biometric investigations on the cnidae of Aegean color morphs of *Actinia equina mediterranea sensu* Schmidt, 1972. *Isr. J. Zool.* **43:** 377–384.

Cutress, C. H. 1955. An interpretation of structure and distribution of cnidae in Anthozoa. *Syst. Zool.* **4:** 120–137.

Denny, M. W., T. L. Daniel, and M. A. R. Koehl. 1985. Mechanical limits to size in wave-swept organisms. *Ecol. Monogr.* **55:** 69–102.

Fautin, D. G. 1988. Importance of nematocysts to actinian taxonomy. Pp. 487–500 in *The Biology of Nematocysts*, D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego, CA.

Fautin, D. G., A. Bucklin, and C. Hand. 1989. Systematics of sea anemones belonging to the genus *Metridium* (Coelenterata: Actiniaria), with a description of *M. giganteum* new species. *Wasmann J. Biol.* **47:** 77–85.

Francis, L. 1973a. Clone specific segregation in the sea anemone *Anthopleura elegantissima*. *Biol. Bull.* **144:** 64–72.

Francis, L. 1973b. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.* **144:** 73–92.

Francis, L. 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. *Biol. Bull.* **150:** 361–376.

Francis, L. 1979. Contrast between solitary and clonal lifestyles in the sea anemone *Anthopleura elegantissima*. *Am. Zool.* **19:** 669–681.

Francis, L. 1985. The ecology and evolution of aggression among sea anemones (Actiniaria). *Natl. Geogr. Res.* **21:** 167–173.

Francis, L. 1988. Cloning and aggression among sea anemones (Coelenterata: Actiniaria) of the rocky shore. *Biol. Bull.* **174:** 241–253.

Geller, J. B., and E. D. Walton. 2001. Breaking up and getting together: evolution of symbiosis and cloning by fission in sea anemones (Genus *Anthopleura*). *Evolution* **55:** 1781–1794.

Gosline, J. M. 1971. Structure and composition of mesogloea in *Metridium*. *J. Exp. Biol.* **55:** 763–774.

Hand, C. 1954. The sea anemones of central California part I. The corallimorpharian and athenarian anemones. *Wasmann J. Biol.* **12:** 345–375.

Hand, C. 1955. The sea anemones of central California part III. The acontiarian anemones. *Wasmann J. Biol.* **13:** 189–251.

Hess, H. C. 1993. The evolution of parental care in brooding spirorbid polychaetes: the effect of scaling constraints. *Am. Nat.* **141:** 577–596.

Hyman, L. H. 1940. *The Invertebrates: Protozoa Through Ctenophora*. McGraw-Hill, New York.

Jaccard, H., and C. K. Wan. 1996. *LISREL Approaches to Interaction Effects in Multiple Regression*. Sage Publications, Thousand Oaks, CA.

Knight, C. G., M. N. Patel, R. B. R. Azevedo, and A. M. Leroi. 2002. A novel mode of ecdysozoan growth in *Caenorhabditis elegans*. *Evol. Dev.* **4**(1): 16–27.

Koehl, M. A. R. 1977. Mechanical diversity of connective tissue of the body wall of sea anemones. *J. Exp. Biol.* **69:** 107–125.

Kramer, A., and L. Francis. 2004. Predation resistance and nematocyst scaling for *Metridium senile* and *M. farcimen*. *Biol. Bull.* **207:** 130–140.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. *Annu. Rev. Ecol. Syst.* **20:** 97–117.

Lehn, H. 1951. Teilungsfolgen und Determination von I-Zellen für die Cnidenbildung bei *Hydra*. *Z. Naturforsch.* **6b:** 388–391.

Longley, R. D. 1984. Axon surface infolding and axon size can be quantitatively related in gastropod molluscs. *J. Exp. Biol.* **108:** 163–177.

Maldonado, R. H., C. San Jose, and B. Martinoya. 1974. Cell size and body weight in some homeotherms and poikilotherms. *Acta Physiol. Latinoam.* **24:** 328–335.

Mariscal, R. N. 1984. Cnidaria: cnidae. Pp. 57–68 in *Biology of the Integument, Vol. I, Invertebrates*. J. Bereiter-Hahn, A. G. Maltolsy, and K. S. Richards, eds. Springer-Verlag, Berlin.

McArdle, B. H. 1988. The structural relationship: regression in biology. *Can. J. Zool.* **66:** 2329–2339.

Munro, H. N. 1969. Evolution of protein metabolism in mammals. Pp. 133–182 in *Mammalian Protein Metabolism*, Vol. 3. H. N. Munro, ed. Academic Press, New York.

Munro, H. N., and J. A. M. Gray. 1969. The nucleic acid content of skeletal muscle and liver in mammals of different body size. *Comp. Biochem. Physiol.* **28:** 897–905.

Niklas, K. J. 1994. *Plant Allometry: The Scaling of Form and Process*. Univ. of Chicago Press, Chicago.

Pearse, V. B., and L. Francis. 2000. *Anthopleura sola*, a new species, solitary sibling species to the aggregating sea anemone, *A. elegantissima* (Cnidaria: Anthozoa: Actiniaria: Actiniidae). *Proc. Biol. Soc. Wash.* **113:** 596–608.

Peters, R. H. 1983. *The Ecological Implications of Body Size*. Cambridge Univ. Press, New York.

Purcell, J. E. 1984. The functions of nematocysts in prey capture by epipelagic siphonophores (Coelenterata, Hydrozoa). *Biol. Bull.* **166:** 310–327.

Rayner, J. M. V. 1985. Linear relations in biomechanics: the statistics of scaling functions. *J. Zool. Lond. A* **206:** 415–439.

Rifkin, J. F. 1991. A study of the spirocytes from the Ceriantharia and Actiniaria (Cnidaria, Anthozoa). *Cell Tissue Res.* **266:** 365–373.

Robson, E. A. 1988. Problems of supply and demand for cnidae in Anthozoa. Pp. 179–207 in *The Biology of Nematocysts*, D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego, CA.

Ryland, J. S., M. M. Brasseur, and J. E. Lancaster. 2004. Use of cnidae in taxonomy: implications for a study of *Acrozoanthus australiae* (Hexacorallia, Zoanthidae). *J. Nat. Hist.* **38:** 1193–1223.

Schmidt, H. 1974. On evolution in the Anthozoa. *Proc. 2nd Int. Coral Reef Symp.* **1:** 533–560.

Schmidt-Nielsen, K. 1984. *Scaling—Why is Animal Size So Important?* Cambridge University Press, Cambridge, MA.

Sebens, K. P. 1981a. The allometry of feeding, energetics, and body size in three sea anemone species. *Biol. Bull.* **161:** 152–171.

Sebens, K. P. 1981b. Reproductive ecology of the intertidal sea anemones *Anthopleura xanthogrammica* (Brandt) and *Anthopleura elegantissima* (Brandt) body size, habitat and sexual reproduction. *J. Exp. Mar. Biol. Ecol.* **54:** 225–250.

Sebens, K. P. 1983. Population dynamics and habitat suitability of the

intertidal sea anemones *Anthopleura elegantissima* and *A. xanthogrammica*. *Ecol. Monogr.* **53:** 405–433.

Sebens, K. P. 1984. Agonistic behavior in the intertidal sea anemone *Anthopleura xanthogrammica*. *Biol. Bull.* **166:** 457–472.

Sebens, K. P. 1987. The ecology of indeterminate growth in animals. *Annu. Rev. Ecol. Syst.* **18:** 371–407.

Sebens, K. P., and G. Laakso. 1978. The genus *Tealia* (Anthozoa: Actiniaria) in the waters of the San Juan Archipelago and the Olympic Peninsula. *Wasmann J. Biol.* **35:** 152–168.

Shick, J. M. 1991. *A Functional Biology of Sea Anemones*. Chapman and Hall, London.

Smith, R. J. 1984. Allometric scaling in comparative biology: problems of concept and method. *Am. J. Physiol.* **246:** R152–R160.

Sokal, R. R., and F. J. Rohlf. 1981. *Biometry: The Principles and Practice of Statistics in Biological Research*, 2nd ed. W. H. Freeman, New York.

Stephenson, T. A. 1929. On the nematocysts of sea anemones. *J. Mar. Biol. Assoc. UK* **16:** 173–201.

Stevenson, R. D., M. F. Hill, and P. J. Bryant. 1995. Organ and cell allometry in Hawaiian *Drosophila:* how to make a big fly. *Proc. R. Soc. Lond. B* **259:** 105–110.

Thomason, J. C. 1988. The allometry of nematocysts. Pp. 575–588 in *The Biology of Nematocysts*, D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego, CA.

Weill, R. 1934a. Contribution a l'étude des cnidaires et de leurs nématocystes, vol. I, recherches sur les nématocystes (morphologie - physiologie - development). *Trav. Sta. Zool. Wimereux* **10:** 1–347.

Weill, R. 1934b. Contribution a l'étude des cnidaires et de leurs nématocystes, vol. II, valeur taxonomique du cnidome. *Trav. Sta. Zool. Wimereux* **11:** 349–701.

Williams, R. B. 1996. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria): statistical parameters and taxonomic relevance. *Sci. Mar.* **60:** 339–351.

Williams, R. B. 1998. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria) II: further studies of differences amongst sample means and their taxonomic relevance. *Sci. Mar.* **62:** 361–372.

Williams, R. B. 2000. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria) III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. *Sci. Mar.* **64**(1)**:** 49–68.

Zamponi, O. M., and F. H. Acuña. 1991. La variabilidad de los cnidocistos y su importancia en la determinacion de clines. *Physis (B. Aires)* **49:** 7–18.

Zar, J. H. 1984. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ.